Abstract of the disclosure

The present invention provides methods, compositions, substrates, and kits useful for analyzing the metabolic activity in cells, tissue, and animals and for screening test compounds for their effect on cytochrome P450 activity. In particular, a one-step and two-step methods using luminogenic molecules, e.g. luciferin or coelenterazines, that are cytochrome P450 substrates and that are also bioluminescent enzyme, e.g., luciferase, pro-substrates are provided. Upon addition of the luciferin derivative or other luminogenic molecule into a P450 reaction, the P450 enzyme metabolizes the molecule into a bioluminescent enzyme substrate, e.g., luciferin and/or luciferin derivative metabolite, in a P450 reaction. The resulting metabolite(s) serves as a substrate of the bioluminescent enzyme, e.g., luciferase, in a second light-generating reaction. Luminescent cytochrome P450 assays with low background signals and high sensitivity are disclosed and isoform selectivity is demonstrated. The present invention also provides an improved method for performing luciferase reactions which employs added pyrophosphatase to remove inorganic pyrophosphate, a luciferase inhibitor which may be present in the reaction mixture as a contaminant or may be generated during the reaction. The present method further provides a method for stabilizing and prolonging the luminescent signal in a luciferase-based assay using luciferase stabilizing agents such as reversible luciferase inhibitors.